Comparison of substrate utilization patterns in males and eumenorrheic females during submaximal exercise

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COMPARISON OF SUBSTRATE UTILIZATION PATTERNS IN MALES AND EUMENORRHEIC FEMALES DURING SUBMAXIMAL EXERCISE.

by

Theodore Zderic

B.S. University of Washington, 1994

presented in partial fulfillment of the requirements

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Approved by:

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Dean, Graduate School

12-8-97

Date

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Comparison of Substrate Utilization Patterns in Males and Eumenorrheic Females During Submaximal Exercise (76 pp.)

Director: Dr. Brent C. Ruby

The purpose of this investigation was to examine substrate oxidation and plasma glucose kinetics in males and regularly menstruating females during submaximal exercise. Five recreationally trained males and females (VO₂peak = 61.7 ± 2.9 vs 48.6 ± 3.0 ml·kg⁻¹·min⁻¹, respectively) performed cycle ergometry exercise for 25 minutes at ~70%VO₂ of their respective lactate threshold (LT), immediately followed by 25 minutes of exercise at ~90%VO₂ LT. Female subjects were tested during the midfollicular (MF) phase (4-6 days after onset of menses) and during the luteal (L) phase (22-27 days after onset of menses). All subjects were tested after an 11 hour fast and within two hours of waking. Carbohydrate and fat oxidation were determined with indirect calorimetry (respiratory exchange ratio and VO₂) while plasma glucose production (Ra) and utilization (Rd) were determined by a primed (30 μmol·kg⁻¹) constant infusion (0.42 μmol·kg⁻¹·min⁻¹) of 6,6-²H-glucose. During exercise at 70% LT (~40-50% VO₂peak), males and females, regardless of menstrual phase, oxidized similar proportions of carbohydrate and fat. At rest and during 70% LT, there were no differences between genders and menstrual phases in plasma glucose Ra and Rd. At 90% LT (~52-62% VO₂peak), males and MF females oxidized similar proportions of carbohydrates and fats and had similar glucose Ra and Rd (μmol·kg lean body mass⁻¹·min⁻¹). However, during the L phase, females tended to oxidize proportionally less carbohydrate and more fat compared to the males (p>0.05) and the MF phase (p<0.05). In accord with the decrease in carbohydrate oxidation at 90%LT during the L phase, plasma glucose Rd was lower than in males and the MF phase (both p<0.05). With the present data, it cannot be determined whether the differences in substrate oxidation are due to increased lipid availability during the L phase or a decreased glycolytic flux. Future studies in this area should employ the muscle biopsy technique to determine the pre-exercise muscle glycogen concentrations. These results suggest that conclusions about gender differences in substrate oxidation and plasma glucose utilization during exercise depend upon the comparison exercise intensity and the menstrual phase of the females subjects.
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Training, traveling, and triathloning with the crew will be missed more than anything, but I’ll be back as much as possible.

Finally, thanks to the beautiful Missoula Valley for her ever-changing yellows and greens, protective hills, and temperate summers.

Have the love. Use the Force.

Go Stampede!
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Chapter One: Introduction

Introduction

In today's society there is an ever growing number of people participating in endurance exercise. The progression of this increased participation has been facilitated by many factors including the rise of running road races twenty years ago, the increased popularity of triathlon in the last decade, and the recent Surgeon General warning about the health risks associated with physical inactivity. In 1984, women's running was popularized by the introduction of the women's marathon into the Olympic Games. Currently, endurance events women commonly compete in include distance running, cycling, and triathlons and events that involve over 12 hours of continuous activity like ultramarathons and ironman triathlons. In 1996, the largest triathlon in Northwestern United States was an all-women's triathlon that consisted of nearly 1000 female competitors. Indeed, women are significantly involved in endurance exercise today.

Although females today are a large component of the endurance athlete population, whether females demonstrate a specific metabolic response to exercise has not been adequately addressed. While several factors including endurance training, cardiorespiratory fitness, exercise intensity, and duration have been demonstrated to have significant effects on the metabolic response to acute exercise, whether or not gender (e.g., hormonal profile) affects exercise metabolism remains inconclusive. If gender differences do
exist in exercise metabolism then this may have implications for defining
gender specific strategies in sport nutrition, training, and competition.

There are obvious differences in physical performance potential
between genders. Males hold most all world records that require strength,
power, speed, and endurance. This gender difference in athletic performance
is attributed to males generally having larger body size, more muscle mass,
larger hearts, greater hemoglobin concentration, and less relative body fat
(Brooks & Fahey, 1985). While these physical differences are well
documented, gender differences in fat and carbohydrate metabolism during
exercise are not.

It has been hypothesized from anecdotal evidence that females do not
experience the extreme fatigue of marathons known as “hitting the wall.”
This extreme fatigue is characterized by liver and muscle glycogen depletion
and hypoglycemia. A typical explanation for this type of empirical
observation is that females utilize relatively more fat and hence spare more
muscle glycogen during prolonged exercise than males. This proposed
increased fat use is usually attributed to a more effective fat metabolism and
to the fact that women generally have relatively more body fat than men.
While females generally have relatively more body fat than males, this may
not dictate endurance performance, because even the leanest male endurance
athletes have ample fat stores to successfully complete ultramarathons and
ironman triathlons. The issue of whether females metabolize fat more
effectively is less conclusive.
Since carbohydrate stores in the human body are much more limited than fat stores, the ability to spare carbohydrate is of great concern to the endurance athlete. Costill et al. (1979) concluded that there were no differences in fat metabolism in trained males and females who had similar VO_{2\text{max}}, training mileage, and muscle fiber composition. In contrast to this finding, Tarnopolsky et al. (1990) concluded that females demonstrate greater fat utilization and less carbohydrate utilization than males during exercise.

Observed differences in substrate utilization between genders are usually attributed to circulating ovarian hormones (Bunt, 1990). It has been shown repeatedly in ovariectomized female and male rats that the ovarian hormone, estradiol has significant effects on substrate utilization during exercise (Hatta et al., 1988; Kendrick et al., 1987; Kendrick & Ellis, 1991). Whether ovarian hormones have an effect on substrate utilization in humans is much more difficult to assess. Due to a constantly changing hormonal milieu of the menstruating female, it is difficult to isolate the effects of select hormones on substrate utilization. Ruby et al. (1997) used amenorrheic (low circulating levels of ovarian hormones) females to assess the effects of acute administration of estradiol on substrate pattern utilization during exercise. They discovered that total carbohydrate and fat oxidation was not affected by the estradiol treatment. However, carbohydrate metabolism was affected as the rate of glucose appearance from the liver and the rate of glucose disposal into the muscle tissue were decreased as a result of the estradiol treatment.
Progesterone which mirrors the estradiol rise and fall during the follicular and luteal phases has been shown to antagonize estradiol effects on substrate use in rats (Hatta et al., 1988). It has been suggested that if indeed estradiol is antagonized by progesterone, the ratio of estradiol to progesterone may be more important than the absolute levels of estradiol and progesterone (Reinke et al., 1972). To determine the combined effects of ovarian hormones (estradiol, progesterone, follicle stimulating, and luteinizing hormone) on substrate metabolism during exercise, females are studied during the follicular and luteal phases of the menstrual cycle. Currently the collective results are equivocal as to whether menstrual phase affects substrate use during exercise. When differences between menstrual phases were determined, exercise during the luteal phase usually elicits lower blood lactate, lower epinephrine (Sutton et al., 1978; Jurkowski et al., 1981), and higher insulin levels (Bonen et al., 1983; Bonen et al., 1991). These changes are suggestive of an effect on carbohydrate metabolism.

If indeed gender and the associated hormonal milieu affect substrate metabolism during exercise, then optimal nutrition, training, and competition for the female athlete may have to be adjusted to be in accord with the ovarian hormonal status. This study was designed with consideration of previous protocols and results to effectively reduce confounding variables and to best detect substrate utilization patterns in males and females.
Problem

The purpose of this study is to determine if there are gender differences in substrate utilization during submaximal exercise. This study will examine and compare the proportions of fat and carbohydrate to total oxidation, and the relative contributions of liver and muscle glycogen stores to total oxidation in active males and females during exercise at 70% and 90% of lactate threshold. In order to explain the observed similarities or disparities, select metabolic regulatory and ovarian hormones will be monitored. The data collected will aid in determining if there are gender differences in nutritional and training guidelines for exercise.

Subproblem

A secondary purpose of this study is to determine if menstrual phase (mid-follicular or luteal) affects substrate utilization during submaximal exercise. This study will examine and compare the proportions of fat and carbohydrate to total oxidation and the relative contributions of liver and muscle glycogen stores to total oxidation in active females during exercise at 70% and 90% of lactate threshold in the mid-follicular and mid-luteal phases. In order to explain the observed similarities or disparities, select metabolic regulatory and ovarian hormones will be monitored. The data collected will aid in determining if exercise training, competition, and nutrition should be adjusted according to the menstrual phase.
Research Hypotheses

Hypothesis One

There will be no difference in the percentage of the total oxidized carbohydrate and fat substrates during exercise between genders.

Justification

Due to the heterogeneity of protocols, the matching procedures, and the general disregard for menstrual status, it is difficult to derive a directional hypothesis from prior research. Several past studies have concluded that females oxidize more fat relative to males (Friedmann & Kindermann, 1989; Froberg & Pedersen, 1984; Jansson et al., 1986; Tarnopolsky et al., 1990; Tarnopolsky et al., 1995). Several other studies have concluded that there are no gender differences in the proportion of oxidized carbohydrates and fats during exercise (Powers et al., 1980; Wallace et al., 1980; Costill et al., 1979; Mendenhall et al., 1995). In all the reviewed studies on gender differences except one (Wallace et al., 1980), the sole criteria for exercise intensity was a set percentage of VO$_2$max. Subjects with the same VO$_2$max can have very different fuel selection patterns at similar exercise intensities if their lactate thresholds (%VO$_2$max) are different (Coggan et al., 1992). When Wallace et al. (1980) had active males and females run at 89% of their respective lactate thresholds they discovered no differences in carbohydrate and fat oxidation.

In two studies where there were no gender differences in substrates oxidized, the male and females were matched on VO$_2$max expressed per kg
body weight (Powers et al., 1980; Costill et al., 1979). Since females generally have less relative muscle mass (i.e., higher fat %), the females matched on \( \text{VO}_2\text{max per kg body weight (ml·kg}^{-1}·\text{min}^{-1}) \) may be expected to be more fit than the males. Although the subjects in the above studies may have had similar \( \text{VO}_2\text{max and similar training this does not ensure similarities in lactate thresholds. Therefore, conclusions about gender differences from these above studies are tenuous because the differences could have simply been due to higher lactate thresholds in the females.}

Since it has been shown fairly conclusively in rats that ovarian hormones can affect carbohydrate metabolism during exercise, the hormonal status of the female subjects should be taken into account in gender comparison studies. Only two reviewed studies (Tarnopolsky et al., 1990; Mendenhall et al., 1995) have controlled for this variable. Lavoie et al. (1987) and Hackney et al. (1994) concluded that the menstrual phase of the female plays a role in substrate selection patterns. Since most of the reviewed studies did not control for the potentially confounding effects of matching subjects and exercise intensity with \( \text{VO}_2\text{max} \), and menstrual status, a directional hypothesis about gender differences in substrate selection cannot be determined.

*Hypothesis Two*
There will be no difference in the percentage of the total oxidized carbohydrate and fat substrates during exercise between luteal and mid-follicular phases of the menstrual cycle.

**Justification**

Collectively, the majority of the literature suggests that there may be slight differences in metabolic responses to exercise between phases of the menstrual cycle. Only one reviewed study has determined a difference in fat and carbohydrate oxidation between menstrual phases (Hackney et al., 1994). Carbohydrate oxidation was higher in the follicular phase at exercise intensities of 35% and 65% of VO₂max. Bonen et al. (1983 & 1991), Jurkowski et al. (1981), Nicklas et al. (1989), and Kanaley et al. (1987) determined that there were no differences in fat or carbohydrate oxidation between menstrual phases. It has been shown in rats (Hatta et al., 1988; Kendrick et al., 1987; Rooney et al., 1993) that estradiol treatment can decrease carbohydrate oxidation and increase fat oxidation. Since circulating estradiol levels are increased in the luteal phase relative to the follicular phase, it may be expected that carbohydrate oxidation in the luteal phase would be decreased. This may be the case if estradiol acts alone on substrate selection or its action is not affected by other ovarian hormones that are in constant flux throughout the cycle. The ovarian hormone, progesterone mirrors the rise in circulating estradiol in the luteal phase and has been shown to suppress the effect of estradiol on carbohydrate metabolism (Hatta et al., 1988). The
reviewed literature in humans is equivocal to whether fat and carbohydrate oxidation is similar between the follicular and luteal phases of the menstrual cycle.

*Hypothesis Three*

There will be a greater relative contribution of plasma derived glucose to total carbohydrate oxidation (Rd glucose/total carbohydrate oxidation) during exercise in females in the midfollicular phase of the menstrual cycle compared to males.

*Justification*

There has only been one published gender comparison abstract investigating the contribution of plasma derived and intramuscular glycogen to total carbohydrate oxidation (Mendenhall et al., 1995). In this study four males and four females in the follicular phase performed 60 minutes of cycle ergometry at 50%VO₂peak. Free fatty acid and glucose stable isotope tracers were infused during exercise to estimate the contribution of intramuscular and extramuscular sources to substrate oxidation. The females used relatively more plasma derived glucose than the males. This is the only gender comparison study that has used stable isotope tracers to compare the relative contributions of intramuscular and extramuscular sources to substrate oxidation.
Hypothesis Four

There will be no difference in the relative contribution of plasma derived glucose to total carbohydrate oxidation (Rd glucose/total carbohydrate oxidation) during exercise in females in the midluteal phase of the menstrual cycle compared to males.

Justification

The relative contribution of plasma derived glucose to total carbohydrate oxidation between males and females exercised during the midluteal phase has not been examined. Since females in the luteal phase have not been examined with the tracer or muscle biopsy technique in a gender comparison study, the direction of this hypothesis cannot be discerned.

Hypothesis Five

There will be no difference in the relative contribution of plasma derived glucose to total carbohydrate oxidation (Rd glucose/total carbohydrate oxidation) during exercise in females between the midfollicular and midluteal phases of the menstrual cycle.

Justification

No study has investigated menstrual phase effects on the relative contributions of hepatic and muscle stores to total carbohydrate oxidation during exercise. Only one study has examined the effects of menstrual phase
on intramuscular glycogen utilization during exercise (Nicklas et al., 1989). This study demonstrated that there were no differences in intramuscular glycogen or total carbohydrate oxidation during exercise. If there were differences in the relative contribution of plasma derived and intramuscular glycogen during exercise, it would be expected that total carbohydrate or intramuscular glycogen would be different between phases. If one of these variables was different between phases and the other was not, then it would be expected that there are differences in relative contribution of plasma derived glucose to total carbohydrate oxidation.

Significance of the Study

In gender and menstrual phase comparison studies, there has been only one published study (Mendenhall et al., 1995) that has used the stable isotope tracer methodology to examine substrate pattern selection during exercise. This study will be the first to investigate gender effects on glucose kinetics in the luteal phase as well as the first study to compare the relative contributions of plasma derived glucose to total carbohydrate oxidation during the follicular and luteal phases.

Most previous gender and menstrual phase comparison studies have arbitrarily selected the exercise intensity to be a set percentage of VO$_2$max. Cureton (1980) suggested that the lactate threshold may be a better indicator of metabolic fitness than VO$_2$max. Different lactate thresholds can have significant effects on exercise metabolism even in subjects with the same
$\text{VO}_2\text{max}$ (Coggan et al., 1992). An example of this effect would be if two subjects who had the same $\text{VO}_2\text{max}$ but their thresholds were 65% and 75% of $\text{VO}_2\text{max}$. If these subjects were exercised at 70% of $\text{VO}_2\text{max}$, one subject would be above threshold and the other would be below resulting in a greater dependence on carbohydrate for the lower threshold subject. To avoid these potentially confounding metabolic effects of the lactate threshold, subjects of this study will be exercised at intensities based on percentages of their respective lactate thresholds. Only one gender comparison study reviewed (Wallace et al., 1980) has used the lactate threshold to control for exercise intensity. Apparently no studies examining menstrual phase effects on substrate selection have controlled for the lactate threshold.

Between-subjects designs that do not have random assignment to groups, like gender comparison studies necessitate the reduction of confounding variables like fitness level differences within and between groups. These differences may mask potential gender effects or give erroneous conclusions. For a repeated measures (i.e., zero between subjects) design like a menstrual phase comparison, the reduction of within group variation is important to obtain more homogenous trial (menstrual phase) responses to exercise. Reduced variation within trials and between subjects improves the power of detecting differences. The use of lactate threshold to control for exercise intensity is used to address these research design concerns of gender and menstrual phase comparison studies.
Rationale for the Study

The investigation of whether gender or menstrual phase affects substrate oxidation is necessary for a more complete understanding of the female physiological response to exercise. Understanding the metabolic parameters affected by ovarian hormones aids in addressing female specific nutritional concerns. If the circulating ovarian hormones affect the selection of fuel sources, this may have implications for exercise training and competition during the menstrual cycle. If menstrual phase and ovarian hormonal levels have an effect on female metabolism this may also have implications for understanding health issues like gestational diabetes and secondary amenorrhea. During pregnancy fuel selection patterns may be in part dictated by the demands of the developing fetus and placenta. The developing fetus rivals the nervous system in plasma glucose oxidation at rest. The results of this study may aid in the understanding of ovarian hormones effect on glucose metabolism which may be important for understanding the patterns of glucose metabolism during pregnancy.

Limitations

i/ Non-randomized sample. The sample will not be randomly selected. Random selection is used to equalize compared groups on extraneous variables. The menstrual phase comparison will use the same females (i.e., repeated measures) thus eliminating confounding variables (e.g., fitness level, fiber type, diet). The between subjects comparison of males and females
will eliminate the confounding variable of fitness level by basing the exercise intensity on a percentage of the lactate threshold.

ii/ Instrumentation. There is inherent error associated with all instrumentation, however, error will be minimized by using trained testers and calibrated equipment.

iii/ Models for substrate use. There are errors inherent (Cobelli et al, 1987) in the non-steady state assumptions used to calculate glucose kinetics during exercise. While these errors may affect the accuracy (i.e., how close to the true value) of the results, the precision (i.e., consistency of results) will be maintained across subjects as the protocol and the constants used in the calculations will be identical for all subjects.

iv/ Muscle glycogen. This investigation will not use the invasive technique of muscle biopsy to determine pre and post exercise glycogen concentrations. Since the existing levels of glycogen concentration in the muscle before exercise can affect carbohydrate use during prolonged exercise, knowing muscle glycogen concentration would aid in explaining observed substrate selection patterns. To minimize a pre-exercise muscle glycogen effect on substrate use, the exercise bout will only be 50 minutes in duration.
Delimitations

i/ Type of subjects. Since the results of this study may have implications for female nutritional needs during exercise, individuals who regularly participate in aerobic exercise will be used for this study. All subjects will have a VO$_2$peak of at least 45 ml·kg$^{-1}$·min$^{-1}$.

ii/ Lipid kinetics. Due to the complexity of free fatty acid infusion and additional significant cost of tracer, free fatty acid and glycerol kinetics will not be determined in this study. Such results would provide valuable information about the source (adipose or intramuscular) of fat use during exercise.

iii/ Specific intensity levels. This investigation will only study 2 levels of exercise intensity. These 2 intensities were chosen because they represent exercise intensities of recreational athletes.

iv/ Specific exercise mode. Cycling will be used as the only mode of exercise in this study. The use of cycle ergometry does not require the movement of the arms thus simplifies infusion and blood sampling logistics. Cycling is also a very common activity for recreational athletes.

v/ Age of the subjects. For testing purposes, only 20-34 year-old subjects will be selected for this study. Aging has been shown to influence fitness and
substrate use during exercise (Sial et al., 1996). Therefore, the use of 20-34 year-old subjects eliminates the potential confounding effects of aging.

vi/ Menstrual status. This investigation will only study the substrate use patterns in eumenorrheic females. Since the determination of substrate use during mid-follicular and luteal phases are sought, females with irregular or amenorrheic cycles will be excluded from this study.

Definition of Terms

Tracer. Labeled metabolite infused to study a metabolite of interest.

Tracee. The metabolite whose turnover is being examined.

Ra and Rd. Rate of appearance (Ra) and rate of disposal (Rd) of a given metabolite into and out of the circulation. These dependent variables will be expressed in the units $\mu$mol$\cdot$min$^{-1}\cdot$kg$^{-1}$.

Isotopic enrichment. Tracer to tracee ratio. Determined with a gas chromatography-mass spectrometer. Used to calculate Ra and Rd.

Substrate. A selected fuel that is oxidized to yield energy.

Respiratory Exchange Ratio (RER). The ratio of VCO$_2$ expired to VO$_2$ consumed. At submaximal intensities, it can be used to determine the proportion of fats and carbohydrates oxidized. Since more oxygen is required to metabolize fat than carbohydrate, a lower RER indicates more fat oxidation. A RER of 0.71 indicates pure fat oxidation and a RER of 1.00 indicates pure carbohydrate oxidation.
Effective volume of distribution. The physiological space (e.g., blood and interstitial fluid) that is assumed to instantaneously mix a given metabolite. 6,6-^H-glucose. A glucose molecule that has the two hydrogens on the number six carbon replaced with two deuteriums. Used as a stable isotope tracer to measure glucose kinetics. A gas chromatography-mass spectrometer can detect the presence of the deuteriums.

VO₂max. The maximal amount of oxygen an individual can consume.

VO₂peak. The maximal amount of oxygen an individual can consume during a specific mode of exercise.

Lactate threshold. The minimal exercise intensity where lactate removal cannot keep pace with lactate production resulting in increased muscle and plasma lactate concentrations. Usually expressed as a percentage of VO₂max or VO₂peak.

Ovarian hormones. Hormones released by the ovaries that are necessary for the maintenance of the menstrual cycle and the development of a fetus (e.g., estradiol, progesterone, follicle stimulating hormone, luteinizing hormone).

Mid-follicular and luteal. Early follicular refers to the phase of the menstrual cycle when an egg begins maturing and circulating estradiol and progesterone are low. Luteal refers to the phase of the menstrual cycle when the endometrium develops and circulating estradiol and progesterone are elevated.

Amenorrheic. The absence of a menstrual cycle characterized by constantly low levels of circulating estradiol and progesterone.
Eumenorrheic. A normally menstruating female with 10-12 menstrual cycles in the previous 12 months.
Chapter Two: Review of Related Literature

Gender comparisons in substrate utilization during exercise

There has been a significant increase in gender comparison studies in the last 20 years. As indicated by Table 2.1, previous investigations into gender differences collectively suggest that untrained females oxidize more lipid than untrained males at a similar percentage of VO$_2$max. However, these differences do not seem to exist between highly trained males and females.

Research design of gender comparison studies

All gender comparison studies reviewed examine substrate utilization at a set percentage of VO$_2$max. The fitness classification of subjects have ranged from untrained and healthy (Mendenhall et al., 1995; Friedmann and Kinderman, 1989) to trained subjects (Costill et al., 1979; Tarnopolsky et al., 1990; Wallace et al., 1980; Powers et al., 1980). Techniques used to assess substrate utilization have included indirect calorimetry, blood metabolite sampling, muscle biopsies, and stable isotope tracer methodology. All reviewed studies except Friedmann & Kinderman (1989) have used indirect calorimetry (RER and VO$_2$) to calculate the proportional contribution of carbohydrates and fats to total energy expended. This technique involves measuring expired gases to determine the ratio of CO$_2$ produced to O$_2$ consumed. Since the oxidation of fat requires more O$_2$ than carbohydrates, this ratio can be used to determine the proportion of substrates oxidized. All reviewed studies have used blood
sampling to examine various metabolites and hormones including blood glucose, lactate, free fatty acids, glycerol, epinephrine, insulin, and growth hormone. Tarnopolsky et al. (1990) and Nygaard et al. (1978) used the muscle biopsy technique to assess glycogen degradation during exercise by measuring intramuscular glycogen concentrations before and after exercise. Mendenhall et al. (1995) used glucose and free fatty acid stable isotope tracers to determine the relative contribution of intramuscular and extramuscular (e.g., liver, adipose) sources to total substrate oxidation.

Matching of female and male subjects in gender comparison studies
Since fitness level alone can have a significant effect on substrate utilization during exercise (Hurley et al., 1986), prior research has most often used similarly trained males and females to best isolate the effects of gender on substrate use. Maximal oxygen consumption (VO₂max) is used in all reviewed gender comparison studies to define cardiorespiratory fitness. Powers et al. (1980), Costill et al. (1979) expressed VO₂max relative to total body weight (ml·kg⁻¹·min⁻¹) to match males and females for cardiorespiratory fitness. Tarnopolsky et al. (1990), Froberg & Pedersen (1984), Bunt et al. (1986) used males and females who had similar VO₂max expressed per kg lean body weight (i.e., fat-free mass). Graham et al. (1986) and Jansson et al. (1986) did not address the fitness level of their subjects. As noted by Froberg & Pedersen (1984), VO₂max expressed per kg body weight may not be the most appropriate method for
### Table 2.1. Summary of investigations into gender differences in substrate utilization during exercise

<table>
<thead>
<tr>
<th>References</th>
<th>Subjects</th>
<th>Design</th>
<th>Results</th>
<th>Conclusions</th>
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</table>
| Tarnopolsky et al., 1990    | 6 M T    | ~65% VO2max run for 15.5 km; matched for VO2max LBW & midfollicular | F RER < M  
F blood glucose > M  
F IMglycogen use < M  
F epinephrine < M  
F insulin > M | Females use relatively more fat and less carbohydrates during prolonged exercise than males with similar VO2max per LBW and training histories. |
| Mendenhall et al., 1995     | 4 M UT   | Cycling @50% VO2peak for 1 hour             | No differences in RER  
No difference in IMtriglyceride use  
FIMglycogen use < M  
F epinephrine < M | Females in the follicular phase rely more on plasma glucose than males for carbohydrate oxidation during moderate intensity exercise. |
| Froberg & Pedersen, 1984    | 7 M Active | Cycling @80% and 90% VO2max to exhaustion similar VO2max LBW | F lasted longer @80% VO2max  
No differences in times to exhaustion @90% VO2max  
F RER < M @80% VO2max  
No differences in lactate@90% | Females use relatively more fat @ 80% VO2max. Differences in times to exhaustion due to > IM glycogen depletion in males as indicated by RER. No differences @90% because of similar lactate responses. |
| Jansson, 1986               | 18 F 15 M | Cycling @65% VO2max for 25 minutes          | F RER < M during exercise & rest  
No differences in blood glucose, FFA during exercise & rest plasma | Although females had a greater relative proportion of fat oxidation, there were no gender differences in metabolite responses. |
| Graham et al., 1986         | 6 amenorrheic F 6 eumenorrheic F 6 M        | 30% VO2max for 20 min, 60% VO2max for 20 min, then cycle to exhaustion @90% VO2max | Ameno F FFA > M @30%VO2max  
No differences in FFA between eumenorrheic F & M  
No differences in RER  
Lactate & noradrenaline all F < M | Metabolic sexual dimorphism exists as well as distinct differences in female subgroups identified by menstrual status. |
| Friedmann & Kinderman, 1989 | 12 M T 12 F T | T group ran @80% VO2max for 14-17 km  
12 M UT 12 F UT | UT F FFA > M  
UT F blood glucose > M  
No exercise differences in T F&M  
At rest, UT & T F GH1 > M | In untrained subjects there is a difference between F M in glucose and FFA metabolism. There were no gender differences in substrate responses to exercise in trained subjects. |
| Costill et al., 1979         | 12 F T 12 M T | 60 min run @70% VO2max; matched on VO2max BW & training | No differences in RER  
No differences in FFA, glycerol responses during exercise | There are no differences in lipid metabolism in trained males and females during submaximal exercise. |
| Wallace et al., 1980        | 10 F T   | 120 min run @~66% VO2max (89% of lactate threshold) | No differences in blood glucose, lactate, glycerol, FFA, ketones during exercise | Trained males and females metabolize fats and carbs similarly during prolonged exercise at comparable relative intensities. |
| Powers et al., 1980         | 4 F T    | 90 min run @65% VO2max matched on VO2max BW & training | No differences in RER  
No differences in lactate responses | This data does not support a gender difference in fat metabolism when subjects are matched by VO2max per kg body weight and training volume. |
matching cardiorespiratory fitness in males and females. Since females typically have less O_2 consuming tissue (i.e., muscle mass) per kg total body weight due to higher percent body fat, females with similar muscle oxidative capacity should have a lower VO_2 max when expressed per kg body weight. Therefore, this would imply that females with similar VO_2 max per kg body weight to males would be relatively more fit than the males. Buskirk and Taylor (1957) suggested that VO_2 max per kg lean body mass may most appropriately measure cardiorespiratory capacity. However, because females often have lower hemoglobin concentration (i.e., lower O_2 capacity, VO_2 max per kg lean body weight may still show lower values in females. According to Cureton (1981), this difference is often non-significant in small group comparisons due to low statistical power.

Selection of exercise intensity in gender comparison studies

Gender comparisons in substrate utilization have been studied during exercise intensities ranging from 30% VO_2 max (Graham et al., 1986) to 90% VO_2 max (Froberg & Pedersen, 1984). Most studies (Bunt et al., 1986; Wallace et al., 1980; Tarnopolsky et al., 1990; Costill et al., 1979; Graham et al., 1986; Jansson et al., 1986) have examined substrate utilization during moderate exercise intensity (60% to 70% VO_2 max). The lactate threshold was used in the study by Wallace et al. (1980) to assign a running velocity that corresponded to 88% of VO_2 at the lactate threshold. The lactate threshold can have significant effects on substrate utilization (Coggan et al., 1992). Coggan et
al. compared substrate oxidation and glucose kinetics in subjects with similar VO$_2$max and dissimilar lactate thresholds. Subjects with lower lactate thresholds demonstrated a significantly greater dependence on carbohydrate oxidation and a decreased reliance on fat oxidation compared to the high threshold group when exercised at a moderate intensity (~50%VO$_2$max). These authors state that the lactate threshold may be a better measure of skeletal muscle oxidative capacity than VO$_2$max. Although lactate threshold has been demonstrated to affect substrate selection during exercise, the overwhelming majority of gender comparison studies have not used the lactate threshold to assign exercise intensities.

Menstrual status of female subjects in gender comparison studies

The substrate utilization patterns of females used in studies by Mendenhall et al. (1995) and Tarnopolsky et al. (1990) were studied during exercise in the follicular phase when circulating ovarian hormones are relatively low. In the study by Graham et al. (1986), substrate use during exercise was examined in normally menstruating and amenorrheic females. Many other studies (Wallace et al., 1980; Friedmann & Kinderman, 1989; Froberg & Pedersen, 1984; Costill et al., 1979; Powers et al., 1980; Jansson et al., 1986) have not described the menstrual condition of their female subjects when they were studied for substrate utilization. In eumenorrheic females, the phase of the menstrual cycle may have independent effects on metabolic responses to exercise (Hackney et al., 1994; Lavoie et al., 1987; Jurkowski et al., 1981). Table
2.2 summarizes several studies investigating menstrual phase effects on substrate use during exercise. Hackney et al. (1994) determined that carbohydrate use was decreased and fat use increased during the luteal phase compared to the follicular phase in trained females who ran at 35% and 65% of VO$_2$\textsubscript{max}. Lavoie et al. (1987) and Jurkowski et al. (1981) determined that the blood lactate response to exercise was greater during the follicular phase than the luteal phase, suggesting an increased dependence on glycolysis during the follicular phase. The disregard for female menstrual condition in gender comparison studies may confound results as this variable may have significant effects on substrate use during exercise.

Results and discussion of gender comparison studies

Costill et al. (1979) investigated substrate utilization in 12 trained females and 13 trained males during treadmill running at 70% VO$_2$\textsubscript{max}. Male and females were matched on VO$_2$\textsubscript{max} per kg body weight and training volume. No differences in fat or carbohydrate oxidation, blood FFA, or glycerol were discovered between males and females. The authors concluded that there were no differences in fat metabolism in similarly trained males and females during submaximal exercise. Powers et al. (1980) using the same criteria for matching males and females found similar results in four trained males and females matched on VO$_2$\textsubscript{max} per kg body weight and training volume. The results of these studies should be considered in light of the matching procedures and the disregard for menstrual status of the female subjects. As
discussed above, females who have similar VO$_2$max per kg body weight to males may be considered to more fit than their male counterparts due to differences in body composition (Cureton, 1981). All of these subjects were running between 30 and 70 miles a week. Incidence of amenorrhea tends to be higher in women who are involved in heavy training. It might be expected that many of these women have low circulating levels of ovarian hormones making these females more endocrinologically similar to males compared to eumenorrheic females.

In contrast to these above studies, Froberg & Pedersen (1984) and Tarnopolsky et al. (1990) found significant differences in fat and carbohydrate metabolism in similarly trained males and females. The female and male subjects of these studies were matched by VO$_2$max per kg lean body weight. Froberg & Pedersen had 6 physically active females and 7 active males exercise to exhaustion at 80% and 90% of VO$_2$max. During the 80% trial, the females performed for significantly longer times than the males. However, there were no differences in time to exhaustion at 90%VO$_2$max. The greater time to exhaustion in the females is attributed to slower glycogen depletion as indicated by a lower RER (i.e., increased fat oxidation) in the females during the exercise. The similarity in time to exhaustion during the 90% VO$_2$max trial is assumed to be due to comparable patterns of lactate accumulation during this intense exercise.
Table 2.2. Summary of studies examining the effects of menstrual phase on substrate utilization during exercise

<table>
<thead>
<tr>
<th>References</th>
<th>Subjects</th>
<th>Design</th>
<th>Results</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonen et al., 1991</td>
<td>8 F</td>
<td>Run for 30 minutes</td>
<td>No differences in RER, blood FFA, lactate, glucose</td>
<td>Lactate similarities between menstrual phases suggest that muscle carbohydrate metabolism during intense exercise is not altered by changes in ovarian steroids.</td>
</tr>
<tr>
<td></td>
<td>eumenorrheics</td>
<td>@40% VO2max then 30 minutes @85% VO2max</td>
<td>Insulin follicular &lt; luteal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>hGH tends to be lower in follicular, not significant, however</td>
<td></td>
</tr>
<tr>
<td>Bonen et al., 1983</td>
<td>5 fasted F</td>
<td>Run for 30 minutes</td>
<td>No differences in lactate, glycerol, hGH, cortisol, RER</td>
<td>Select metabolic responses are affected by menstrual phase.</td>
</tr>
<tr>
<td></td>
<td>6 glucose-fed F</td>
<td>@40% VO2max then 30 minutes @85% VO2max</td>
<td>Glucose-fed F luteal FFA &lt; follicular</td>
<td></td>
</tr>
<tr>
<td>Jurkowski et al., 1981</td>
<td>9 F</td>
<td>33%, 66%, 90% VO2max cycle ergometry</td>
<td>No differences in VO2, VO2, HR</td>
<td>Aerobic capacity is not affected by menstrual phase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Follicular lactate &gt; luteal @ 90%</td>
<td>Higher lactate in follicular phase due to production and not disposal.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No difference in lactate disposal</td>
<td></td>
</tr>
<tr>
<td>Nicklas et al., 1989</td>
<td>6 F</td>
<td>70% VO2max until exhaustion</td>
<td>Time to exhaustion tended to be &gt; in luteal phase (p&lt;0.07)</td>
<td>Exercise performance and muscle glycogen content are enhanced during luteal phase. Athlete performance may be affected by phases of the menstrual cycle.</td>
</tr>
<tr>
<td></td>
<td>moderately trained</td>
<td></td>
<td>IM glycogen repletion &gt; in luteal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No differences in blood gluc, lactate</td>
<td></td>
</tr>
<tr>
<td>Lavoie et al., 1987</td>
<td>7 F</td>
<td>90 min@63% VO2max</td>
<td>Decrease in blood glucose in luteal @ 70 and 90 minutes</td>
<td>The difference in blood glucose between phases suggests involvement of gonadotrophic and/or gonadal hormones in metabolism during exercise.</td>
</tr>
<tr>
<td></td>
<td>active</td>
<td>after 24 hour diet of low carbs</td>
<td>Lactate follicular &gt; luteal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cortisol luteal &gt; follicular</td>
<td></td>
</tr>
<tr>
<td>Kanaley et al., 1992</td>
<td>6 amenorrheics</td>
<td>Run @60% VO2max for 90 min tested in early &amp; late follicular, &amp; luteal</td>
<td>No differences in hGH response to exercise</td>
<td>GH levels and substrate utilization are independent of both menstrual phase and status. Amenorrhea does not result in metabolic consequences during prolonged exercise by influencing substrate utilization.</td>
</tr>
<tr>
<td></td>
<td>7 eumenorrheics</td>
<td>VO2max for 90 min tested in early &amp; late follicular, &amp; luteal</td>
<td>No difference in fat or carbo use</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All T</td>
<td></td>
<td>E2 did not increase during exercise in amenorrheics</td>
<td></td>
</tr>
<tr>
<td>Dombovy et al., 1987</td>
<td>8 F</td>
<td>Cycle ergometry VO2max test.</td>
<td>No differences in VO2max, exercise duration, HRmax, max ventilation,</td>
<td>Although ventilatory parameters are altered by the menstrual cycle, there is no overall effect on maximal exercise performance.</td>
</tr>
<tr>
<td></td>
<td>UT</td>
<td></td>
<td>cardiac output, ventilatory thresh. luteal RER &lt; follicular</td>
<td></td>
</tr>
<tr>
<td>Hackney et al., 1994</td>
<td>9 F</td>
<td>Running @ 35%,60%, 75% VO2max for 10 min in each intensity</td>
<td>Carbo use luteal @35%,65%&lt; follic</td>
<td>The phase of the menstrual cycle in eumenorheic women does influence metabolic substrate usage during low-to-moderate intensity exercise.</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td></td>
<td>Fat use luteal @35%,65%&gt; follic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No differences @75% VO2max</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations*: BW = body weight; F = females; FFA = free fatty acids; hGH = human growth hormone; M = males; IM = intramuscular; RER = respiratory exchange ratio (VCO2/VO2); T = trained; UT = untrained; VO2max = maximum O2 uptake
In the study by Tarnopolsky et al. (1990), six trained males and females were matched on VO\textsubscript{2}max per kg lean body weight and training volume. Two days before exercise testing all subjects consumed similar diets to minimize the effect of glycogen stores on substrate utilization. The females were exercise tested in the midfollicular phase to control for the potential effect of menstrual phase on substrate use. During treadmill running at 65% of VO\textsubscript{2}max (~90 minutes) males utilized a significantly greater proportion of carbohydrates for oxidation than females, as indicated by RER and muscle biopsy data. The authors concluded that trained females use relatively more fat and less carbohydrates during exercise than trained males with similar VO\textsubscript{2}max per kg lean body mass. While this data controlled for a number of variables including cardiorespiratory fitness (i.e., matching for VO\textsubscript{2}max per kg lean body weight), training volume, menstrual phase, and diet, the potential differences in the lactate threshold between groups was not controlled for. As discussed above the percentage of VO\textsubscript{2}max where this threshold occurs can have significant effects on carbohydrate metabolism during exercise (Coggan et al., 1992). Another explanation for the results of Tarnopolsky et al. and Froberg & Pedersen could simply be that the females had higher lactate thresholds than the males, resulting in a relatively less dependence on carbohydrate. In a study (Wallace et al., 1980) that did control for the lactate threshold, trained males and females who exercised at 88% of lactate threshold did not demonstrate any differences in carbohydrate or fat
metabolism as determined by RER, and blood glucose, lactate, FFA, and glycerol.

In a review article on gender differences in substrate utilization during exercise, Ruby & Robergs (1994) concluded that the literature collectively suggests that gender plays a role in substrate selection in untrained but less so in trained subjects. The results of Friedmann & Kinderman (1989) may be in accordance with this conclusion. These investigators examined gender differences in 48 total trained and untrained males and females. The trained group ran at 80% VO$_2$max for 14-17 km and the untrained group ran at 75% VO$_2$max for 10 km. The males and females being compared had similar lactate thresholds. Blood glucose and FFA were significantly higher in untrained females compared to untrained males. There were no observed differences in blood glucose and FFA in the trained males and females during exercise. The authors concluded that their study does not present any conclusive data to indicate that endurance-trained persons show gender differences in lipid metabolism. They also suggest that the increased blood FFA observed in untrained females may be explained by increased mobilization of FFA from intramuscular fat depots. However, static plasma metabolite concentrations alone do not allow for determination of substrate uptake or utilization. Static plasma concentrations are a function of the rate of appearance of the metabolite into the circulation and the rate of disposal of the metabolite into the tissues. The increased blood FFA seen in females could be due to three different possibilities: increased FFA mobilization into
the blood, decreased disposal into the tissues, or a combination of both. Depending on which case is correct, substrate utilization may or may not be affected by gender. The use of stable isotope tracers would have allowed for the determination of substrate use. As RER data was not gathered during exercise, total carbohydrate and fat oxidation cannot be discerned from this study.

In a recent study using glucose and FFA tracers (Mendenhall et al. 1995), four untrained males and females (in follicular phase) exercised for one hour at 50%VO₂peak on a cycle ergometer. These authors determined that there were no gender differences in the percentages of fat and carbohydrate oxidized during exercise. However, the relative contribution of plasma derived glucose to total carbohydrate oxidation was greater (and muscle glycogen contribution was less) in females than males. This is the first study to suggest that individual components (hepatic glucose production and muscle glycogenolysis) of carbohydrate metabolism may be affected by gender.

Effects of ovarian hormones on substrate selection

Differences in substrate utilization between gender or menstrual phase are usually attributed to differences in circulating ovarian hormones (Bunt, 1990). The ovarian hormones, estradiol and progesterone have been demonstrated to have significant effects on carbohydrate and fat metabolism in exercising rats (Hatta et al., 1988; Rooney et al., 1993; Kendrick et al., 1987; Ellis et al., 1994). Kendrick et al. determined that oophorectomized rats treated with
estradiol spared myocardial glycogen utilization during 2 hours of treadmill running. Rooney et al. (1993) also reported a decrease in hepatic and muscle glycogen utilization in male rats treated with estradiol. Hatta et al. (1988) determined that ovariectomized female rats treated with 17β-estradiol relied less on carbohydrate oxidation than the rats treated with 17β-estradiol and progesterone. The results of Hatta et al. are probably more applicable to a true in vivo situation as estradiol is usually not present as the lone ovarian hormone in females with intact ovaries.

While there have been several studies using rat models to determine the effects of estradiol and progesterone on substrate utilization, there apparently is only one human study (Ruby et al., 1996) that has attempted to isolate the effect of estradiol on substrate utilization during exercise. Isolating the effects of ovarian hormones on substrate utilization in human females is difficult due to the ever changing hormonal milieu of the menstruating female. As mentioned above the effects of estradiol on carbohydrate metabolism may be suppressed by the presence of progesterone (Hatta et al., 1988). Due to ethical and subject concerns, estradiol and progesterone producing ovaries cannot be excised from human females. To best isolate the effects of estradiol on substrate utilization patterns during exercise in humans, Ruby et al. (1996) determined the effect of acute transdermal estradiol administration on glucose and fat metabolism in amenorrheic females. Amenorrheic females are known to have abnormally low levels of circulating estradiol and progesterone. Therefore differences in substrate use
in these females may be ascribed to the estradiol treatment. Total fat and carbohydrate oxidation as assessed by RER data was not significantly affected by the estradiol treatment, however, hepatic glucose production and muscle glucose uptake, as measured by the stable isotope tracer technique, were significantly decreased during the estradiol treatment. As suggested by the results of Mendenhall et al. (1995) above, ovarian hormones may differentially affect individual components of carbohydrate metabolism (i.e., plasma glucose utilization).
Chapter Three: Methodology

Setting

All physiological testing took place in the Human Performance Laboratory, McGill Hall #121, The University of Montana.

Subjects

A group of 5 males and 6 eumenorrheic females served as subjects for this investigation. The sample was comprised of recreational athletes aged 23-33 years with a VO2max of at least 45 ml·kg⁻¹·min⁻¹. The subjects that volunteered to participate in this study completed a University of Montana IRB-approved informed consent form. Height, weight, percent body fat, cycling VO₂ peak, and cycling lactate threshold were determined before completing one, two-staged submaximal cycling trial for the males and one, two-staged submaximal cycling trial during the mid follicular phase and one trial during the mid-luteal phase for the females. The two exercise intensities for the two-stage submaximal trials were set at 70% and 90% of the VO₂ at the lactate threshold.

Descriptive Data

Height, weight, age, menstrual status, and training habits

Data was collected to determine the participants height, weight, age, menstruation status, and training habits. All female subjects were asked to
record their days of menses and their morning oral temperature for two months prior to all exercise trials on a provided calendar in order to accurately predict the time of mid-follicular and mid-luteal phases. None of the females were using oral birth control during the study.

Body composition

Body fat and lean body mass was assessed by hydrostatic weighing at residual lung volume. Residual lung volume was calculated using a Helium dilution technique. Percent body fat was calculated from body density using age/gender specific equations (Lohman, 1992).

Exercise testing

For all metabolic testing a TEEM 100 (Aerosport Inc., Ann Arbor, MI) metabolic system equipped with a medium-flow (10-120 l·min⁻¹) pneumotach was used to analyze expired concentrations of oxygen and carbon dioxide and expired volume. Prior to each cycle test, the metabolic system was gas calibrated with known concentrations of O₂ and CO₂. Flow rate was calibrated using a 3 l syringe before each exercise test according to the manufacturer (Aerosport Operators Manual, 1993). Heart rate was continuously monitored using a telemetry chest strap heart rate monitor for all testing (Polar, Port Washington, NY). All cycling tests took place using the subject's bicycle fixed to a Schwinn Velodyne ergometer for control of power output.
**Peak VO₂ cycling test**

Data were collected every 20 seconds for VO₂ (l·min⁻¹ and ml·kg⁻¹·min⁻¹) and RER. The warm-up period was approximately five minutes. The testing protocol began with three 4-minute steady-state stages of increasing power outputs (males - 100W, 175W, 250W; females - 75W, 125W, 175W). Immediately after the third stage, the power output was increased 25 Watts every minute until volitional exhaustion. The criteria for VO₂ peak was a plateau in VO₂ or a RER>1.1.

**Lactate threshold test**

In order to appropriately prescribe exercise intensities for the two-stage submaximal exercise trial, a lactate threshold test was performed. Before the test, an indwelling venous catheter was placed in an antecubital vein for blood sampling during exercise. The exercise protocol consisted of an initial workload (100 W for males, 50 W for females) for the first minute followed by an increase in power output of 25 W every minute until the females and males reached 100W and 200W, respectively. The power output was increased 15 W every minute thereafter until ~90%VO₂max. Blood (~1-2 ml) was sampled during the last 15 seconds of each 1-minute stage. Five hundred μl of blood was immediately deproteinized in 1 ml of 7% perchloric acid and frozen at -20° C until determination of blood lactate by an enzymatic assay (Lowry, 1971). Power output at lactate threshold was defined as the last workload before a curvilinear increase in plasma lactate concentration was
observed. Three of the females performed this test in the luteal phase first and two performed the test in the follicular phase first. Menstrual phase has been shown not to affect the %VO2peak at which the anaerobic threshold occurs (Dombovy et al., 1987).

Two-stage submaximal exercise trial with 6,6-^{2}H-glucose

Before the two-stage submaximal test, an indwelling teflon catheter (18-20 gauge) was inserted into an antecubital vein of each arm. One catheter was used to prime the body and constantly infuse 6,6-^{2}H-glucose in 0.45% saline. A 60-ml syringe depressed by a Harvard infusion pump (Cambridge Isotopes Laboratories, Woburn, MA) was used to infuse approximately 3.0 μmol·kg^{-1}·min^{-1} of 6,6-^{2}H-glucose for the first 10 minutes followed by 80 minutes of constant infusion at a rate of 0.42 μmol·kg^{-1}·min^{-1}. The catheter in the contralateral arm was used for blood sampling and was kept patent with a continuous saline drip. After the 80 minutes of constant infusion to obtain isotopic equilibrium, the subject cycled at a power output corresponding to ~70% of the VO_{2} at their lactate threshold for 25 minutes immediately followed by 25 minutes of cycling at ~90% of the VO_{2} at their lactate threshold. Blood samples were taken before priming (to assess background 6,6-^{2}H-glucose), 10 minutes, 5 minutes and immediately before exercise for resting glucose kinetics, and every 5 minutes during exercise for determination of 6,6-^{2}H-glucose, glucose, lactate, and insulin concentrations (Figure 3.1). Expired gases were monitored during the last 5 minutes of each of the two
stages for VO$_2$ and RER using the metabolic system mentioned above. To reduce the possibility that test familiarity would have an effect on the metabolic response, three of the five female subjects performed the two-stage submaximal test in mid-follicular phase first and the other two females performed the test during the mid-luteal phase first. Subjects were asked to refrain from eating 10 hours and exercising 36 hours prior to the two-stage submaximal exercise trial. All female subjects were instructed to eat a similar diet before each of their two trials and all subjects submitted a two-day dietary record before the two-stage submaximal exercise trials. All subjects ate at least 4 g·kg$^{-1}$ carbohydrate per day.

**Figure 3.1** Blood sampling protocol for two-stage submaximal exercise trial (minutes)

<table>
<thead>
<tr>
<th>priming dose</th>
<th>constant infusion of 6,6-$^{2}$H-glucose</th>
<th>EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 μmol/kg-min</td>
<td>0.42 μmol/kg-min</td>
<td>[ L \quad 70%; LT \quad \quad 95%; LT \quad L ]</td>
</tr>
<tr>
<td>-100</td>
<td>-90</td>
<td>-10</td>
</tr>
</tbody>
</table>

| G | G | G | G | G | G | G | G | G | G | La | La | La | La |

| Ins | Ins | Ins |

| E2 |

3 | SAMPLE VOLUMES (mL) | 3 | 7 | 5 | 3 | 3 | 3 | 5 | 7 | 3 | 3 | 3 | 5 | 7 |

**Abbreviations:** G = glucose and 6,6-$^{2}$H-glucose; La = plasma lactate; Ins = insulin; E2 = estradiol

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Metabolite and hormone assays

Glucose and lactate assays

Plasma from the necessary blood samples for glucose and lactate were obtained through centrifugation of the clotted blood samples and then frozen at -20°C. The supernatant was frozen at -20°C until determination of blood glucose and lactate concentrations. Glucose and lactate concentrations from the two-stage submaximal trial were analyzed with a blood glucose and lactate analyzer (YSI, Yellow Springs, OH). All samples were analyzed in duplicate.

Hormones

Plasma from the necessary blood samples for hormone analysis were obtained through centrifugation of the clotted blood samples and then frozen at -20°C. Insulin (kit #TKIN2 - sensitivity of 1.5 μIU/mL) and estradiol (kit #KE2D1 - sensitivity of 1.4 pg/mL) were assayed for with commercially available double antibody radio-immuno assay kits (Diagnostic Products Corp., Los Angeles, CA). All samples were analyzed in duplicate.

Isotopic enrichment

The ratio of 6,6-²H-glucose to unlabeled glucose (isotopic enrichment) was determined by forming a pentaacetate derivative of glucose and using a gas-chromatography mass spectroscopy technique as described by Wolfe (1992). In brief, the peak abundances of ions m/e 200 for glucose, m/e 201 and m/e 202...
for 6,6-$^2$H-glucose were monitored for calculating plasma glucose enrichment. A Hewlett-Packard 5980 Plus gas chromatograph-mass spectrometer with a packed column was used to separate the pentaacetate derivative from other organic compounds. The pentaacetate derivative was fragmented with electron ionization of 70 keV and the ions of mass 200, 201, and 202 were selectively monitored for abundances to calculate the enrichment of the plasma glucose.

**Calculation of glucose kinetics: Ra, Rd, glycogen and plasma glucose utilization**

Glucose rates of appearance (Ra) and disposal (Rd) from the circulation were calculated with the Steele (1959) equation using non-steady state assumptions during the last 5 minutes of the two exercise intensities.

\[
Ra = \frac{F - (VC) dE/dt}{E} \\
Rd = Ra - (V) dC/dT
\]

where

- F is the rate of infusion of the isotope (\(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\)),
- V is the effective volume of distribution for glucose (\(\text{ml}\cdot\text{kg}^{-1}\)),
- C is the concentration of glucose (\(\mu\text{mol}\cdot\text{ml}^{-1}\)),

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E is the enrichment of glucose (6,6-$^2$H-glucose : glucose ratio),

dE/dT is the instantaneous change in enrichment at a given time,

dC/dT is the instantaneous change in glucose concentration at a given time.

Example calculations are provided below:

$$Ra = \frac{0.42 \ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} - 100 \ \text{ml}\cdot\text{kg}^{-1} (4.44 \ \mu\text{mol}\cdot\text{ml}^{-1}) (-0.004/5 \ \text{min})}{0.03}$$

$$= 25.8 \ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$$

$$Rd = 25.8 \ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} - 100 \ \text{ml}\cdot\text{kg}^{-1} (-0.1 \ \mu\text{mol}\cdot\text{ml}^{-1}/5 \ \text{min}) = 27.8 \ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$$

The time series smoothing technique of spline fitting was used to best approximate the change in isotopic enrichment (dE/dt) and change in glucose concentration (dC/dt) during exercise. The relative contribution of blood glucose to total carbohydrate oxidation was calculated using the average glucose Rd from the last 5 minutes of each exercise intensity as the rate of blood glucose oxidation. The oxidation of Rd glucose is between 96-100% at 50% VO2max (A. Jeukendrup, unpublished observation). The difference between total carbohydrate oxidation and Rd determined the minimal rate of intramuscular glycogen oxidation. For the calculation of Ra, the effective volume of distribution for glucose was set at 100 ml·kg$^{-1}$. 

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Research design and statistical procedures

The descriptive variables VO_{2}max (ml·kg⁻¹·min⁻¹ and ml·kg⁻¹LBW·min⁻¹) and %VO_{2}max at lactate threshold were analyzed with independent sample t-tests to compare males and females. Dependent sample t-tests were used to analyze differences between phases for single level factors (estradiol, body weight). All other dependent variables were analyzed based on a series of a priori planned comparisons (Table 3.1) (Shavelson, 1988). All planned comparisons were analyzed with SuperAnova statistical package (Abacus Inc, Berkeley, CA). The level of significance was set at an overall experimental alpha of 0.05.

Table 3.1. Outline of a priori planned comparisons

1) Blood metabolites and hormones - comparison of follicular and luteal phases at rest, 20-25, and 45-50 minutes for the variables blood glucose, lactate, and insulin. (3 level, within factor) FF vs FL p<0.033. (3 level, between factor) M vs FF and M vs FL p<0.017.

2) Substrate selection - a) RER, % of total kcal from carbohydrate, and % of total kcal from fat using mean from time points 20 and 25 minutes of each exercise intensity. (2 level, within factor) FF vs. FL, p<0.025. (2 level, between factor) M vs FF and M vs FL p<0.013.
3) Source of oxidized carbohydrates - % of total oxidized carbohydrates from intramuscular glycogen and % of total oxidized carbohydrates from plasma derived glucose using the mean from time points 20 and 25 minutes of each intensity. (2 level, within factor) FF vs. FL, p<0.025. (2 level, between factor) M vs FF and M vs FL p<0.013.

Calculations for Type I error

1) FF vs. FL, (3 level, within factor) p<0.033 because the P(Type I error)=1-(1-x)^{3/2}=0.05. x=0.033 for 3 comparisons (rest, low, high)

2) FF vs FL (2 level, within factor) p<0.025 because the P(Type I error)=1-(1-x)^2 =0.05. x=0.025 for 2 comparisons.

3) M vs FF and M vs FL (3 level, between factor) p<0.017 because the P(Type I error)=1-(1-x)^{3/2}=0.05. x=0.033 for 3 comparisons and since M compared twice (vs FF and FL) P(Type I error)=1-(1-x)^2 =0.033. x=0.017

4) M vs FF and M vs FL (2 level, between factor) p<0.013 because the P(Type I error)=1-(1-x)^2=0.05. x=0.025 for 2 comparisons and since M compared twice (vs FF and FL) P(Type I error)=1-(1-x)^2 =0.025. x=0.013.
Chapter Four: Results

Subject characteristics

All of the subjects (n=11) of this study except one female (cyclist) were recreational triathletes who had participated in at least one triathlon in the last 12 months and were training for swimming, cycling, and running during the study. All expressions of cycle ergometer maximal oxygen consumption (l·min⁻¹, ml·kg⁻¹·min⁻¹, ml·kg⁻¹ lean body mass·min⁻¹) were significantly greater in the males (p<0.01). The males were taller (p<0.02) and had a lower body fat percentage (p<0.02). While maximal heart rate and the % VO₂peak at the lactate threshold were lower in females these were not significant (p=0.36, p=0.055, respectively). All five females used in the male versus luteal phase female comparison were used in the menstrual phase comparison. One of the five females used in the male versus mid-follicular comparison was not used in the menstrual phase comparison because of a failed luteal phase trial.

Table 4.1. Physical characteristics of males (n=5) and regularly menstruating females (n=5). Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>MALES</th>
<th>FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.0 ± 2.0</td>
<td>24.0 ± 1.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.9 ± 1.8</td>
<td>165.3 ± 2.3*</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>68.0 ± 2.8</td>
<td>63.2 ± 3.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>8.8 ± 1.4</td>
<td>16.4 ± 1.8*</td>
</tr>
<tr>
<td>Maximum Heart Rate (b·min⁻¹)</td>
<td>191 ± 4.2</td>
<td>186 ± 2.5</td>
</tr>
</tbody>
</table>
The physical characteristics of the 5 regularly menstruating females examined in the mid-follicular and mid-to-late luteal phases are presented in Table 4.2. Four of the five females used in the menstrual comparison were used in the gender comparison component of this study. All of the females were menstruating regularly for at least five months prior to the study and none of the females were using oral contraceptives during the study according to the responses of the menstrual history questionnaire. Cyclical changes in serum estradiol were used to confirm the presence of a changing hormonal milieu consistent with a menstruating female. Anticipated rises in oral temperature were only confirmed in three of five subjects. Inadequate recording of morning oral temperature was most likely responsible for the lack of detection in the two females who discerned no increase in oral temperature, because both had elevated estradiol levels during the luteal phase.
Table 4.2. Physical characteristics of menstrual phase comparison subjects n=5. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.8± 1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.9 ± 3.0</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>59.3 ± 4.2</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>15.2 ± 1.5</td>
</tr>
<tr>
<td>Maximum Heart Rate</td>
<td>184.4 ± 1.3</td>
</tr>
<tr>
<td>(b·min⁻¹)</td>
<td></td>
</tr>
<tr>
<td>VO₂peak (l/min)</td>
<td>2.87 ± 0.21</td>
</tr>
<tr>
<td>VO₂peak (ml·kg⁻¹·min⁻¹)</td>
<td>48.9 ± 1.3</td>
</tr>
<tr>
<td>% VO₂peak @ lactate threshold</td>
<td>60.1 ± 2.2</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>13.2 ± 0.2</td>
</tr>
<tr>
<td>Length of menstrual cycle</td>
<td>28 ± 0.5</td>
</tr>
<tr>
<td>(days)</td>
<td></td>
</tr>
</tbody>
</table>

Expression of exercise intensity during the two-stage subthreshold trial

The planned intensity for the low intensity workload was 70% of the VO₂ at the lactate threshold (% VO₂ @ LT) and the high intensity was planned for 90% of the VO₂ at the lactate threshold. There were no differences in the % VO₂ @ LT, HR, and the %HRmax during the low intensity between the males and the follicular phase females nor between the males and the luteal phase
females (Table 4.3). The luteal phase females tended to have lower heart rates than the males during the low intensity workload but this was not significant.

Since the lactate threshold of the males occurred at a higher percentage (p=0.055) of their VO$_2$ peak, the intensity when expressed as % VO$_2$ peak was significantly higher for the males compared to both phases of the females during both low and high intensity workloads.

There were no significant differences in %HRmax between the males and both phases of the females, although during the high intensity workload, the luteal phase females tended to work at a lower %HRmax than the males (p=0.019). The follicular females worked at a significantly lower percent of their lactate threshold (p=0.011) than the males during the high intensity workload. Any deviation from the planned intensity was most likely due to insufficient prediction of the watts required to elicit the appropriate oxygen consumption and electronic error of associated with the cycle ergometer. During the high workload, the females in both phases had significantly lower heart rates than the males.
Table 4.3. Workload intensities for the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male-Lo</td>
<td>Follic-Lo</td>
</tr>
<tr>
<td>% VO₂ @ LT</td>
<td>68.7 ± 0.9</td>
<td>67.3 ± 2.7</td>
</tr>
<tr>
<td>% VO₂ peak</td>
<td>47.3 ± 1.5</td>
<td>41.8 ± 1.6*</td>
</tr>
<tr>
<td>HR</td>
<td>129 ± 5.0</td>
<td>124 ± 5.9</td>
</tr>
<tr>
<td>%HRmax</td>
<td>67.5 ± 1.3</td>
<td>66.8 ± 3.1</td>
</tr>
</tbody>
</table>

Different from males  *p<0.013 (α=0.05), **p<0.01

There were no significant differences in oxygen consumption between the follicular and luteal phases at the low and high workloads (Table 4.4), although the luteal high workload tended to be higher (p=0.07). However, heart rate was significantly lower at the higher workload during the luteal phase.

Table 4.4. Workload intensities for the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follic-Lo</td>
<td>Lut-Lo</td>
</tr>
<tr>
<td>% VO₂ @ LT</td>
<td>69.8 ± 2.4</td>
<td>69.5 ± 2.2</td>
</tr>
<tr>
<td>% VO₂ peak</td>
<td>41.8 ± 1.6</td>
<td>41.7 ± 1.7</td>
</tr>
<tr>
<td>HR</td>
<td>121.6 ± 4.7</td>
<td>119.9 ± 2.4</td>
</tr>
<tr>
<td>%HRmax</td>
<td>66.0 ± 2.9</td>
<td>65.0 ± 1.5</td>
</tr>
</tbody>
</table>

Different from follicular  *p<0.025 (α=0.05), **p<0.01
Indirect calorimetry and substrate oxidation

According to respiratory exchange ratios (RER), there were no significant differences in the percentage of calories derived from carbohydrate and fat oxidation between the males and the females (Table 4.5). However, the percentage of fat oxidized was greater in the luteal phase females than males and the percentage of calories derived from carbohydrate were lower, but this did not reach statistical significance (p=0.025 > 0.013 (α=0.05)).

Table 4.5. Carbohydrate and fat oxidation during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male-Lo</td>
<td>Folic-Lo</td>
</tr>
<tr>
<td>RER</td>
<td>0.819 ± 0.010</td>
<td>0.826 ± 0.018</td>
</tr>
<tr>
<td>%CHO</td>
<td>40.5 ± 3.4</td>
<td>42.7 ± 6.4</td>
</tr>
<tr>
<td>%FAT</td>
<td>59.5 ± 3.4</td>
<td>57.3 ± 6.4</td>
</tr>
</tbody>
</table>

Rates of carbohydrate and fat oxidation, and the percentage of calories derived from carbohydrate and fat at the lower workload were similar during the follicular and luteal phases (Table 4.6). At the higher workload, however, fat oxidation (g FAT/min) was increased significantly by 21% in the luteal phase compared to the follicular phase and the percentage of calories derived from fat (53.3% vs. 44.7%) were higher during the luteal phase. Correspondingly, carbohydrate oxidation (g CHO/min) was reduced by 10% during the high workload of the luteal phase and the percentage of calories derived from carbohydrates was lower in the luteal phase (46.7% vs 55.3%).

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Table 4.6. Carbohydrate and fat oxidation during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Low Intensity</th>
<th>High Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follic-Lo</td>
<td>Lut-Lo</td>
</tr>
<tr>
<td>RER</td>
<td>0.831 ± 0.015</td>
<td>0.830 ± 0.021</td>
</tr>
<tr>
<td>%CHO</td>
<td>44.6 ± 5.1</td>
<td>44.1 ± 7.3</td>
</tr>
<tr>
<td>%FAT</td>
<td>55.4 ± 5.1</td>
<td>55.9 ± 7.3</td>
</tr>
<tr>
<td>g CHO/min</td>
<td>0.70 ± 0.12</td>
<td>0.69 ± 0.14</td>
</tr>
<tr>
<td>g FAT/min</td>
<td>0.33 ± 0.02</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>kcal/min</td>
<td>5.74 ± 0.42</td>
<td>5.71 ± 0.38</td>
</tr>
</tbody>
</table>

Different from follicular *p<0.025 (α=0.05)

Plasma glucose concentrations

There were no significant differences in glucose concentrations (Table 4.7) between the males and females, although glucose concentrations tended to be higher in the luteal and follicular females (4.39 and 4.32 mM, respectively) compared to the males (3.94 mM) at the low intensity (p=0.075 and p=0.043 > 0.017 (α=0.05), respectively). Glucose concentrations were slightly but significantly lower (~0.13 mM) in the luteal phase during rest, low intensity, and high intensity workloads (Table 4.8).

Table 4.7. Glucose concentrations during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>4.19 ± 0.20</td>
<td>4.23 ± 0.09</td>
<td>4.42 ± 0.10</td>
</tr>
<tr>
<td>Low</td>
<td>3.94 ± 0.33</td>
<td>4.32 ± 0.15</td>
<td>4.39 ± 0.15</td>
</tr>
<tr>
<td>High</td>
<td>4.49 ± 0.21</td>
<td>4.49 ± 0.12</td>
<td>4.50 ± 0.16</td>
</tr>
</tbody>
</table>
Table 4.8. Glucose concentrations (mM) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>4.28 ± 0.08</td>
<td>4.42 ± 0.10*</td>
</tr>
<tr>
<td>Low</td>
<td>4.23 ± 0.17</td>
<td>4.39 ± 0.15**</td>
</tr>
<tr>
<td>High</td>
<td>4.38 ± 0.16</td>
<td>4.50 ± 0.16*</td>
</tr>
</tbody>
</table>

Different from follicular *p<0.033 (α=0.05), **p<0.01

*Plasma glucose kinetics*

Only three of the five luteal trials used for all other gender comparisons were available for comparison of plasma glucose kinetics because of implausible results from gas chromatography-mass spectrometry analysis. Since there was a significant difference in body composition (i.e., body fat percentage), the glucose rates of appearance (Ra) and disappearance (Rd) were expressed and compared as umol/kg/min and umol/kg lean body mass/min. Glucose concentrations were stable at rest in all subjects, therefore glucose rates of appearance and disappearance (i.e., glucose turnover) into and out of the circulation were equal (i.e., Ra=Rd at rest). During rest and the low intensity workload, there were no differences in the glucose Ra or Rd between the males and females regardless of the units used to express the kinetics (Tables 4.9-4.13).

At the high intensity workload, Ra (umol/kg/min) tended to be lower (24.0 vs. 30.0) in the follicular females (p=0.026 > 0.017 (α=0.05) (Table 4.9)) versus the males. However, when expressed as Ra (umol/kg lean body mass/min) follicular females were similar to males (28.8 vs. 32.9, p=0.16)
(Table 10). Rd (umol/kg/min) also tended to be lower (25.4 vs. 29.8) in the follicular females (p=0.056 > 0.017 (α=0.05)) (Table 4.11), but when Rd was expressed as umol/kg lean body mass/min follicular females and males were similar (30.4 vs. 32.7 (Table 4.12)). At the high intensity, regardless of the units used to express glucose turnover, the luteal phase females had significantly lower rates of glucose turnover compared to the males (Tables 4.9-4.13).

Table 4.9. Glucose rates of appearance (Ra) into the circulation (umol/kg/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>12.1 ± 0.2</td>
<td>12.9 ± 0.6</td>
<td>12.5 ± 0.2</td>
</tr>
<tr>
<td>Low</td>
<td>19.3 ± 1.9</td>
<td>17.1 ± 1.7</td>
<td>16.6 ± 1.3</td>
</tr>
<tr>
<td>High</td>
<td>30.0 ± 3.7</td>
<td>24.0 ± 2.7</td>
<td>20.0 ± 3.2**</td>
</tr>
</tbody>
</table>

Different from males *p<0.017 (α=0.05), **p<0.01

Table 4.10. Glucose rates of appearance (Ra) into the circulation (umol/kg lean body mass/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>13.4 ± 0.4</td>
<td>15.4 ± 0.5</td>
<td>14.7 ± 0.2</td>
</tr>
<tr>
<td>Low</td>
<td>21.1 ± 1.9</td>
<td>20.5 ± 2.0</td>
<td>19.5 ± 1.5</td>
</tr>
<tr>
<td>High</td>
<td>32.9 ± 3.9</td>
<td>28.8 ± 3.4</td>
<td>23.5 ± 3.9*</td>
</tr>
</tbody>
</table>

Different from males *p<0.017 (α=0.05)
Table 4.11. Glucose rates of disappearance (Rd) out of the circulation (umol/kg/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>12.1 ± 0.2</td>
<td>12.9 ± 0.6</td>
<td>12.5 ± 0.2</td>
</tr>
<tr>
<td>Low</td>
<td>17.9 ± 1.7</td>
<td>16.3 ± 1.4</td>
<td>15.8 ± 1.2</td>
</tr>
<tr>
<td>High</td>
<td>29.8 ± 3.3</td>
<td>25.4 ± 2.6</td>
<td>19.4 ± 6.5**</td>
</tr>
</tbody>
</table>

Different from males *p<0.017 (α=0.05), **p<0.01

Table 4.12. Glucose rates of disappearance (Rd) (umol/kg lean body mass/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>13.4 ± 0.4</td>
<td>15.4 ± 0.5</td>
<td>14.7 ± 0.2</td>
</tr>
<tr>
<td>Low</td>
<td>19.6 ± 1.7</td>
<td>19.5 ± 1.7</td>
<td>18.6 ± 1.6</td>
</tr>
<tr>
<td>High</td>
<td>32.7 ± 3.5</td>
<td>30.4 ± 3.1</td>
<td>22.7 ± 4.2**</td>
</tr>
</tbody>
</table>

Different from males *p<0.017 (α=0.05), **p<0.01

Table 4.13. Glucose rates of clearance (Rc) (ml/kg/min) during the two-stage subthreshold trial gender comparison. n=5 males, n=5 follicular females, n=3 luteal females. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>2.92 ± 0.14</td>
<td>3.05 ± 0.11</td>
<td>2.73 ± 0.05</td>
</tr>
<tr>
<td>Low</td>
<td>4.67 ± 0.60</td>
<td>3.81 ± 0.46</td>
<td>3.45 ± 0.36</td>
</tr>
<tr>
<td>High</td>
<td>6.82 ± 1.04</td>
<td>5.72 ± 0.71</td>
<td>4.18 ± 0.95**</td>
</tr>
</tbody>
</table>

Different from males *p<0.017 (α=0.05), **p<0.01

For the glucose kinetics menstrual phase comparison, data from only 4 of the 5 females were available due to implausible results obtained from gas
chromatography mass spectrometry analysis. During rest and the low workload, there were no differences in glucose kinetics between the luteal and follicular phases. At the high workload there were no differences in Ra (umol/kg/min) between follicular and luteal phases (Figure 4.1). However, Rd and clearance (Rc = Rd/[glucose]) were significantly lower during the high workload of the luteal phase (Figures 4.2 and 4.3).

**Figure 4.1.** Glucose rates of appearance (Ra) into the circulation during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.
Figure 4.2. Glucose rates of disappearance (Rd) out of the circulation during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error. *p<0.033 (α=0.05)

Figure 4.3. Glucose clearance rate (Rc) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error. **p<0.01
**Plasma lactate concentrations**

There were no differences in plasma lactate concentrations between males and either the follicular or luteal phase females during rest and the low intensity (Table 4.14). However, at the high workload, males had significantly higher plasma lactate concentrations than both phases of the females.

**Table 4.14.** Plasma lactate concentrations (mM) during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>1.52 ± 0.14</td>
<td>1.51 ± 0.19</td>
<td>1.22 ± 0.10</td>
</tr>
<tr>
<td>Low</td>
<td>2.04 ± 0.24</td>
<td>2.26 ± 0.12</td>
<td>1.68 ± 0.14</td>
</tr>
<tr>
<td>High</td>
<td>4.32 ± 0.67</td>
<td>3.24 ± 0.34*</td>
<td>2.48 ± 0.41*</td>
</tr>
</tbody>
</table>

Different from males *p<0.017 (α=0.05)

There were no differences in plasma lactate accumulation during rest and the low intensity between the follicular and luteal phases (Table 4.15). At the high workload, there was a greater plasma lactate concentration during the follicular phase (p<0.017).

**Table 4.15.** Plasma lactate concentrations (mM) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>1.49 ± 0.20</td>
<td>1.22 ± 0.10</td>
</tr>
<tr>
<td>Low</td>
<td>2.01 ± 0.15</td>
<td>1.68 ± 0.14</td>
</tr>
<tr>
<td>High</td>
<td>3.08 ± 0.39</td>
<td>2.48 ± 0.41*</td>
</tr>
</tbody>
</table>

Different from follicular *p<0.033 (α=0.05)
**Insulin**

The luteal phase females had significantly higher insulin concentrations than males during rest, low and high workloads (Table 4.16). The follicular females had significantly higher insulin concentrations during rest and at the high workloads, while insulin tended to be higher during the low workload (3.88 vs 4.72 μIU/ml, \( p=0.023 > 0.017 (\alpha=0.05) \)).

There were no significant differences in plasma insulin concentrations between follicular and luteal phases (Table 4.17), although insulin tended to be lower at the high intensity during the luteal phase (4.11 vs 4.73 μIU/ml, \( p=0.089 > 0.033 (\alpha=0.05) \)).

**Table 4.16.** Insulin concentrations (μIU/ml) during the two-stage subthreshold trial gender comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Rest</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>4.19± 0.42</td>
<td>5.45± 0.61</td>
<td>5.17± 0.33</td>
</tr>
<tr>
<td>Low</td>
<td>3.88± 0.51</td>
<td>4.72± 0.32</td>
<td>4.72± 0.16</td>
</tr>
<tr>
<td>High</td>
<td>3.49± 0.39</td>
<td>4.95± 0.36</td>
<td>4.11± 0.52</td>
</tr>
</tbody>
</table>

**Table 4.17.** Insulin concentrations (μIU/ml) during the two-stage subthreshold trial menstrual phase comparison. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>5.22± 0.63</td>
<td>5.07± 0.16</td>
</tr>
<tr>
<td>Low</td>
<td>4.47± 0.37</td>
<td>4.72± 0.16</td>
</tr>
<tr>
<td>High</td>
<td>4.73± 0.53</td>
<td>4.11± 0.52</td>
</tr>
</tbody>
</table>
Estimation of plasma glucose, muscle glycogen, and fat to total substrate oxidation

While the follicular phase females tended to contribute more of their blood glucose to total carbohydrate oxidation (Table 4.18) there were no significant differences between males and follicular females at either intensity, nor between males and luteal phase females. However, when expressed as contribution of plasma glucose to total kilocalories, the follicular females depended more on plasma glucose at low and high workloads (8.8 vs 11.7%, 11.0 vs 14.2%, respectively, p<0.01 for both workloads) (Figure 4.4). There were no significant differences between luteal phase females and males in the contribution of plasma glucose to total kilocalories.

Table 4.18. Percentage of blood glucose contribution to total carbohydrate oxidation. Expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Follicular</th>
<th>Luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>22.3 ± 2.8</td>
<td>29.2 ± 4.8</td>
<td>24.4 ± 3.5</td>
</tr>
<tr>
<td>High</td>
<td>21.5 ± 2.5</td>
<td>26.4 ± 2.8</td>
<td>21.6 ± 4.2</td>
</tr>
</tbody>
</table>
Figure 4.4. Relative contributions of fat, blood glucose, and muscle glycogen to total substrate oxidation during the high workload gender phase comparison. Blood glucose contribution to total energy expenditure is greater in follicular females (p<0.01).

Plasma glucose contributed 37% of the total carbohydrate oxidized during the luteal phase and 33% during the follicular phase at both intensities (Figure 4.5). Muscle glycogen oxidation conversely contributed 63% and 67% to total carbohydrate oxidation, respectively. There were no significant differences between phases in the percent contributions. When expressed as the percent contribution of plasma glucose to total kilocalories expended, plasma glucose during the follicular phase contributed slightly more to energy expenditure than during the luteal phase at high workload (16.4% vs 14.2%, p=0.032>0.025 (α=0.05)). There were no significant differences in muscle glycogen oxidation between follicular and luteal phases (2.66 vs 2.54 kcal/min, at the high workload, respectively, p=0.38), nor was there a difference at the low workload (1.67 vs 1.75 kcal/min, p=0.55).
Figure 4.5. Relative contributions of fat, blood glucose, and muscle glycogen to total substrate oxidation during the high workload menstrual phase comparison. Blood glucose utilization is higher in follicular phase (p<0.023).
Chapter Five: Discussion

The purpose of this study was to compare carbohydrate and fat metabolism in males and females during exercise below their respective lactate thresholds. A secondary purpose of this study was to determine if females metabolize carbohydrate and fat similarly during the follicular and luteal phases of the menstrual cycle. The subjects were studied while working at exercise intensities (~70%LT and ~90%LT) below the lactate threshold for two purposes: 1) to insure that the respiratory exchange ratio (e.g., VCO$_2$) represented tissue CO$_2$ production and was not influenced by acid buffering by bicarbonate pools, and 2) to maintain the potential effects of reproductive hormones on the glucoregulation capacity of pancreatic hormones. At higher intensities (e.g., above LT), the pancreatic hormones apparently play little role in controlling rates of hepatic glucose production and the factors that control glucose production at high intensities remain unidentified (Coggan et al., 1997).

Substrate oxidation in males and females

The results of this study suggest that conclusions about gender differences in substrate oxidation depend upon the exercise intensity and the menstrual phase during which the comparison females are tested. The decision to compare males and females with respect to their lactate threshold instead of the more traditional approach of selecting exercise intensities with
respect to VO_{2\text{max}} was based on the observation that the lactate threshold is a better measure or predictor of substrate oxidation. Subjects with the same VO_{2\text{max}} and different thresholds can have vastly different rates of carbohydrate and fat oxidation during moderate intensity exercise (Coggan et al, 1992). Kanaley et al (1995) has demonstrated that moderately trained runners and marathon runners with significantly different VO_{2\text{max}} values of 52 and 65 ml/kg/min have similar respiratory exchange ratios (RER) at intensities just above and below their respective lactate threshold. Similarly, Pereira & Freedson (1997) have demonstrated that trained runners and moderately trained runners (69 and 58 ml/kg/min, respectively) have similar RER values at ~88% of the VO_{2} at the lactate threshold. Due to the apparent association of the lactate threshold with substrate oxidation (i.e., RER), this study was designed to have males and females exercise at intensities relative to their respective lactate thresholds to minimize between subject variability and maximize statistical power.

The males and follicular females of this study oxidized similar proportions of fat and carbohydrate at both intensities examined in this study. These results are in contrast to the findings of Froberg & Pedersen (1984), Jansson (1986), Tarnopolsky et al (1990), and Tarnopolsky et al (1995), but are in accord with the results of Costill et al (1977), Wallace et al, and Mendenhall et al (1995). The studies that were in conflict with the results of this study did not control for the lactate threshold and the explanation for the increased fat
oxidation in the female subjects may have been due to a greater level of metabolic fitness (i.e., higher lactate threshold).

The discrepancy between these results and Tarnopolsky et al (1990) who controlled for diet may have been due to the effect of the macrocomposition of the pre exercise trial diets. Aside from the 10 hour fast before all trials, the diet of the subjects was not controlled. The males tended to consume more kilocalories relative to body weight (45 vs 29 kcal per kg, p=0.06). However, the percentage of calories derived from carbohydrates was significantly greater in the females (73 vs 54%, p<0.01) and consequently there were no differences in carbohydrate intake (5.8 vs 6.2 g/kg, females vs males p>0.6) between males and females. Interestingly, Tarnopolsky et al (1995) demonstrated that follicular females who increased their carbohydrate intake from ~55% to ~75% of total kilocalories ingested had no significant effects on muscle glycogen concentration or utilization during exercise. Lambert et al (1994) demonstrated that a high fat diet (70% fat, 7% carbohydrate) compared to a low fat diet (74% carbohydrates, 12% fat) significantly reduces RER values during moderate intensity exercise. These are extreme diets and whether the differing diets between the males and females in the present investigation affected RER values remains unknown. If the composition of the diet did indeed favor an increased carbohydrate oxidation in the females, this would explain the disparity between the results of Tarnopolsky et al (1990 and 1995) and the present male vs. follicular comparison. However, the differences in diets between the males and the luteal females improves the likelihood that
luteal phase females oxidize proportionally less carbohydrate than males, since there is decreased carbohydrate oxidation (p=0.025 > 0.017 (α=0.05)) in the luteal females even though they consumed a greater proportion of their kilocalories as carbohydrates. Future gender comparison studies should control for the macrocomposition of the diet to eliminate this speculation.

**Gender comparison of blood glucose production (Ra) and utilization (Rd)**

During rest and the low intensity workload there were no differences between males and females in glucose rates of appearance (Ra) or rates of disappearance (Rd). At the high workload the conclusion about gender differences in glucose Ra and Rd depends upon two factors 1) the units used to express these parameters and, 2) the menstrual phase of the females. While Ra and Rd (umol/kg/min) appeared to be lower in the follicular females, when expressed relative to lean body mass (i.e., µmol/kg LBM/min), the males and follicular females were similar. Since glucose uptake by the adipose tissue during exercise is most likely negligible compared to the glucose Rd of active skeletal musculature, excluding adipose tissue likely provides a better representation of the glucose kinetics in two groups who are significantly different in body composition. Since the follicular females had similar rates of glucose utilization (Rd) compared to the males and their absolute workload (e.g., total kilocalories expended) was lower, they relied significantly more on blood glucose for their total energy expenditure at both the low and the high workloads (11.5% vs 8.8% and 14.2% vs 11.0%,
respectively, p<0.001). The follicular females also tended to have greater relative contributions of blood glucose to total carbohydrate oxidation at the low and high workloads (29.2% vs 22.3%, p=0.07 and 26.4% vs 21.5%, p=0.17, respectively). This finding is in agreement with the only other study that has compared males and follicular females with glucose stable isotope tracers (Mendenhall et al., 1995). They determined that blood glucose contributed a higher percentage to total carbohydrate oxidation than in males (28 vs 20%, p<0.001). Conversely, muscle glycogen contribution to total carbohydrate oxidation was less in females (72 vs 80%, p<0.001). They speculated that the two fold greater epinephrine levels observed in their male subjects were responsible for the greater contribution of muscle glycogen to total carbohydrate oxidation. Since epinephrine concentrations were not measured in the present investigation, conclusions about this mechanism cannot be established.

Regardless of the units used to express glucose kinetics, luteal females demonstrated suppressed glucose Ra and Rd compared to the males. Males and luteal females contribute similar proportions of their muscle glycogen and blood glucose to total carbohydrate oxidation. However, as there were only 3 luteal females available for these comparisons, conclusions about males and luteal females are tenuous at this point.
Gender comparison of insulin, glucose, and lactate

In agreement with Tarnopolsky et al (1990), males had lower insulin concentrations during rest, low, and high workloads regardless of menstrual phase. It may be possible that this decreased insulin concentration was due to greater male epinephrine levels as determined by Tarnopolsky et al (1990), Mendenhall et al (1995), Graham et al (1986), and Nygaard (1981) and suggested by significantly greater lactate concentration (4.3 vs 3.2 mM follicular, vs 2.5 mM luteal, p<0.01) in the males at the high workload. Despite the differences in insulin concentrations, there were no differences between males and females in glucose concentrations which is in agreement with Jansson (1986) who exercised males and females for 25 minutes at 65% VO_{2max}. While Tarnopolsky et al (1990) observed lower blood glucose concentrations in males, it is possible that the males of that study had lower lactate thresholds and an associated greater glucose Rd (Coggan et al., 1992).

Substrate oxidation across the menstrual cycle

The primary finding from this component of the study is that carbohydrate oxidation is decreased and fat oxidation is increased during the luteal phase of the menstrual cycle which is in agreement with Hackney et al. (1994) and Dombovy et al. (1987), and suggested by others data (Hirata et al., 1986). Hackney et al. used respiratory exchange ratio (RER) and VO$_2$ to measure carbohydrate and fat oxidation (@35%, 60%, 75% VO$_{2max}$) during the midfollicular and midluteal phases in 9 women with a mean VO$_{2max}$ of 46.0
ml/kg/min during treadmill running. At both 35% and 60% VO2max, the mean RER was significantly lower during the luteal phase and thus carbohydrate oxidation was reduced and fat oxidation increased during the luteal phase. While there was no statistical difference in carbohydrate or fat oxidation, RER was still slightly higher during follicular phase at 75% VO2max. Hackney et al. (1994) concluded that exercise at 75% VO2max requires predominantly carbohydrate regardless of phase. Dombovy et al., also observed decreases in RER during an incremental VO2max test in the luteal phase. However, in accord with the results of the current study, there were no differences in RER at the lower workloads (40-50% VO2peak) but differences in RER at intensities above 67% VO2max.

In agreement with our results and Dombovy et al. (1987), Hirata et al. (1986) also determined that in females with similar VO2max values to our subjects, exercise at 40% VO2max (i.e., also the low workload of the present investigation) elicited similar mean RER values during follicular and luteal phases (0.788 vs 0.783, respectively) while at 70% VO2max their mean RER was lower during the luteal phase (0.849 to 0.827, respectively, p>0.05) although not statistically lower. This discrepancy in statistical results between their study and the present investigation may be explained by the statistics used to compare the phase responses. Hirata et al. (1987) used a statistical paired t-test to detect differences while in this study the more powerful a priori planned comparison was used to detect differences. Kanaley et al. (1992) and Nicklas et al. (1989) also concluded that there were no differences in substrate oxidation
between follicular and luteal phases. The subjects of Kanaley et al (1992) were of greater aerobic fitness than the subjects of the current investigation (48 vs. 57 ml/kg/min VO$_{2\text{max}}$), but still had consistently (however p>0.05) lower RER values for 90 minutes of exercise in the luteal phase. The subjects of Nicklas et al (1989) had significantly lower RER values during the luteal phase at rest (0.86 to 0.71, p<0.05) but during 90 minutes of exercise at the relatively high intensity of 70% VO$_{2\text{max}}$ there were no apparent differences.

**Menstrual phase and blood glucose utilization**

While previous menstrual phase studies have relied exclusively on RER to determine substrate oxidation, this is the first study to have studied blood glucose uptake during exercise across the menstrual cycle. In accord with the 10% decrease in carbohydrate oxidation (RER and VO$_2$) during the luteal phase, there was a concomitant 11% decrease in glucose uptake from the circulation. Assuming that 100% of the glucose Rd is oxidized at 50% VO$_{2\text{max}}$ (A. Jeukendrup, unpublished observations) and menstrual phase does not affect the proportion of Rd that is oxidized, glycogen utilization must have also been decreased by a similar percentage during the luteal phase.

Ruby et al. (1997) also observed a decrease in glucose Rd in amenorrheics treated with estradiol. In contrast to this study, there were no significant differences in total carbohydrate or fat oxidation as determined by RER. They also determined a lower glucose Ra which they attributed to the suppressive effect of estradiol on gluconeogenesis previously demonstrated.
in rats (Ahmed & Bailey, 1981; Sladek et al., 1974). The differences in the Ra and RER responses between Ruby et al (1997) and the current investigation are most likely due to the low levels of circulating progesterone in amenorrheics. Indeed, progesterone is known to antagonize estradiol suppression of gluconeogenesis (Ahmed & Bailey, 1974) and estradiol promotes fat oxidation in rats (Hatta et al, 1990). As suggested by Reinke et al (1972) and Bisdee et al (1988) the ratio of estradiol to progesterone may be the most important variable for dictating the metabolic response. Since this study did not investigate the level of circulating progesterone, no conclusions about this ratio can be made.

The decrease in the glucose uptake (Rd) during the luteal phase at the higher workload may have been due to the associated decrease in insulin concentration ($p=0.09$) and or decreased insulin sensitivity during the luteal phase (Diamond et al., 1989; Elkind-Hirsch et al., 1993). As insulin facilitates Rd during exercise (Gao et al, 1994) decreased insulin concentration and or action may in part explain the decreased glucose Rd at the higher workload during the luteal phase.

The greater lactate concentration in the follicular phase is also in accord with the greater carbohydrate oxidation and glucose Rd observed during the follicular phase. McCracken et al. (1994) also reported that the lactate during recovery from ~30 minutes of exhaustive exercise was higher (8.7 vs. 5.4 mM, $p<0.05$) during the follicular phase. Since Jurkowski et al. (1981) determined that there were no differences in the clearance of lactate between phases.
during exercise, the increased lactate concentration at the high workload during the follicular phase was most likely due to a greater glycolytic flux and not due to reduced lactate removal.

It is possible that the shift from carbohydrate oxidation to fat oxidation during the luteal phase was due to an increase in free fatty acid (FFA) availability. Reinke et al (1972) observed that resting FFA levels were significantly higher during the mid luteal phase compared to the follicular and ovulation phases. Elevated estradiol (178 vs 71 nM, p=0.09) and or increased growth hormone concentrations (Nicklas et al, 1989) in the luteal phase may be mediating increased lipolytic action. This proposed mechanism for decreasing carbohydrate oxidation would be in agreement with Hargreaves et al. (1991) who found a decrease in glucose uptake by leg muscles when FFA levels were increased from 0.6 mM to greater than 1.0 mM with the infusion of a triglyceride emulsion (Intralipid) and heparin. Bracy et al. (1995) also demonstrated a decreased blood glucose uptake by increasing FFA levels from 0.3 to 1.0 mM in dogs. During the lower workload of this present study, FFA availability may not have been as limiting because the demand for substrate was not as great. Bonen et al. (1983) and Bonen et al. (1991) reported no menstrual phase differences in FFA levels during light (35-40% \( VO_{2\text{max}} \)) and heavy exercise (83-85% \( VO_{2\text{max}} \)) which were intensities below and above the intensity (~53% \( VO_{2\text{max}} \)) where we have observed differences in fat and carbohydrate oxidation. Without FFA concentrations and kinetics, the
proposed mechanism for decreased blood glucose utilization and carbohydrate oxidation is speculative.

Another explanation for the decreased carbohydrate oxidation would be decreased muscle glycogen stores. Rates of muscle glycogenolysis and carbohydrate oxidation have been shown to be directly related to muscle glycogen concentration in the rat hindquarter (Hargreaves, 1997). Therefore, if pre-exercise muscle glycogen was greater during the follicular phase this may explain the observed increase in carbohydrate oxidation and lactate concentration. However, to avoid differences in pre-exercise muscle glycogen, subjects were asked to replicate their diet two days preceding each trial and to refrain from exercise 36 hours before each trial. Post-hoc comparison of diet records between follicular and luteal phases indicate that there were differences in energy intake (30.7 vs 29.3 kcal/kg, respectively, p=0.7) or carbohydrate intake (5.9 vs 5.2 g/kg CHO, respectively, p=0.3), between the two phases of the menstrual cycle.

While energy balance (i.e., dietary intake minus energy expenditure) was assumed to be similar between phases due to similarities in dietary intake and physical activity this does not preclude differences in resting energy expenditure. Bisdee et al. (1989) observed a significantly greater resting energy expenditure of 130 kcal/day during the luteal phase, while Eck et al (1997) and Piers et al (1995) have reported that resting energy expenditure is similar during the luteal and follicular phases. The gross marker of energy balance, body weight, was significantly higher (60.5 to 60.0 kg, p<0.05) during the luteal
phase suggesting maintenance of positive energy balance. However, water retention may have been responsible for the increased body weight. If so, then it may be possible that during the luteal phase the females were actually in negative energy balance (provided they retained one kilogram or more of water) and the decreased carbohydrate oxidation could have been due to a relative depletion of muscle glycogen. If reduced energy intake of carbohydrates was responsible for the decline in carbohydrate oxidation we would expect to see a positive relationship between carbohydrate intake and oxidation between our subjects. In contrast, there is a strong negative relationship ($r = -0.96$, $p<0.01$) between carbohydrate intake and oxidation during the luteal phase. That is, during the luteal phase the females who consumed less carbohydrates per kilogram oxidized proportionally more carbohydrates during the high workload. Furthermore, in both of the studies that have compared resting muscle glycogen, muscle glycogen concentration was higher during the luteal phase (Hackney, 1990; Nicklas et al., 1989). Nicklas et al (1989) determined that repletion was greater after depleting exercise during the luteal phase even when dietary intake (as in this study) was slightly lower during the luteal phase (1691 vs 1813 kcal/day $p>0.05$). Nevertheless, future studies in this area should measure resting energy expenditure during the menstrual phase of interest and adjust dietary intake accordingly.

There are apparently no studies that have suggested elevated carbohydrate oxidation during the luteal phase. While this does not prove
the converse, from a metanalysis point of view, if there were indeed no differences between phases and bias did not influence experimental designs and results, then there should be a similar number of studies that find decreased carbohydrate oxidation and the converse, increased carbohydrate oxidation during the luteal phase.

Conclusions

In conclusion, these results suggest that there are menstrual phase differences in substrate oxidation patterns in recreational female athletes. Conclusions about gender differences in substrate oxidation depend upon, 1) the exercise intensity and, 2) menstruation status of the female. With the present data, it cannot be determined whether the differences in substrate oxidation are due to increased lipid availability during the luteal phase or a decreased glycolytic flux. Future research in this area should employ stable isotope FFA tracers to determine the source of the increased fat oxidation (i.e., plasma FFA and/or intramuscular triglycerides) witnessed during the luteal phase. The muscle biopsy technique should also be utilized to determine pre-exercise glycogen concentrations. Studies that examine the effects of diet, training, and exogenous substances on substrate utilization should recognize and take into account the menstrual status of their female subjects.
References


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